PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44*bis*)

Applicant's or agent's file reference GC840-PCT	**FOR FURTHER ACTION**	See item 4 below
International application No. PCT/US2005/014182	International filing date *(day/month/year)* 25 April 2005 (25.04.2005)	Priority date *(day/month/year)* 26 April 2004 (26.04.2004)
International Patent Classification (8th edition unless older edition indicated) See relevant information in Form PCT/ISA/237		
Applicant GENENCOR INTERNATIONAL, INC.		

1. This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 *bis*.1(a).

2. This REPORT consists of a total of 10 sheets, including this cover sheet.

   In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.

3. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☒	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

4. The International Bureau will communicate this report to designated Offices in accordance with Rules 44*bis*.3(c) and 93*bis*.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44*bis* .2).

	Date of issuance of this report 20 February 2007 (20.02.2007)
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No. +41 22 338 82 70	Authorized officer Simin Baharlou e-mail: pt09.pct@wipo.int

Form PCT/IB/373 (January 2004)

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2005/014182	25.04.2005	26.04.2004

International Patent Classification (IPC) or both national classification and IPC
INV. G01N33/50

Applicant
GENENCOR INTERNATIONAL, INC.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:	Date of completion of this opinion	Authorized Officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	see form PCT/ISA/210	Schalich, Juliane Telephone No. +49 89 2399-8915

Form (PCT/ISA/237) (Cover Sheet) (April 2005)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2005/014182

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1 (b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

      ☒ a sequence listing

      ☐ table(s) related to the sequence listing

   b. format of material:

      ☒ on paper

      ☒ in electronic form

   c. time of filing/furnishing:

      ☐ contained in the international application as filed.

      ☐ filed together with the international application in electronic form.

      ☒ furnished subsequently to this Authority for the purposes of search.

3. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of

☐ the entire international application

☒ claims Nos. 1-47 (in part)

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international search *(specify)*:

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed *(specify)*:

☒ no international search report has been established for the whole application or for said claims Nos. 1-47 (in part)

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

- ☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
- ☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
- ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rules 13*ter*.1(a) or (b).

☐ a meaningful opinion could not be formed without the tables related to the sequence listings; the applicant did not, within the prescribed time limit, furnish such tables in electronic form complying with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions, and such tables were not available to the International Searching Authority in a form and manner acceptable to it.

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in electronic form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See Supplemental Box for further details

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees, the applicant has, within the applicable time limit:

   ☐ paid additional fees

   ☐ paid additional fees under protest and, where applicable, the protest fee

   ☐ paid additional fees under protest but the applicable protest fee was not paid

   ☒ not paid additional fees

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1-47 (in part)

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	
	No:	Claims	1-47
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-47
Industrial applicability (IA)	Yes:	Claims	1-47
	No:	Claims	

2. Citations and explanations

   see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item IV
Lack of unity of invention

The only subject matter common to all claims is a population-based identification of CD4+ T-cell epitopes within various proteins of interest.
D1 (p 37, last par. till p 42, par. 2 and examples 1-6, claims 1-10) discloses methods for assessing immune response profiles of animal populations, methods for ranking the relative immunogenicity of a first protein and at least one additional protein, including methods, where the second protein is a variant of the first one, and methods allowing for the determination of the level of exposure of said test population to said test protein.

In the light of the prior art, the present application therefor provides (at least) 18 solutions to the problem which can be defined as the provision of methods for identification of T-cell epitopes within defined test proteins population wide. The solutions, i.e. the test proteins, are different, so that the technical relationship between them required by Rule 13 PCT is lacking and the requirement for unity of invention is not fulfilled.

A search of all the different subjects would have caused major additional searching efforts. Consequently, only the first subject was searched.

Re Item V
Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:
D1: WO 03/073068 A (GENENCOR INTERNATIONAL, INC; HARDING, FIONA, A) 4 September 2003 (2003-09-04)
D2: STICKLER M ET AL: "AN IN VITRO HUMAN CELL-BASED ASSAY TO RANK THE RELATIVE IMMUNOGENICITY OF PROTEINS" TOXICOLOGICAL SCIENCES, ACADEMIC PRESS, SAN DIEGO, FL,, US, vol. 77, no. 2, February 2004 (2004-02), pages 280-289
D3: HERMAN A E ET AL: "Determination of glutamic acid decarboxylase 65 peptides presented by the type I diabetes-associated HLA-DQ8 class II molecule identifies an

immunogenic peptide motif." JOURNAL OF IMMUNOLOGY (BALTIMORE, MD. : 1950) 1 DEC 1999, vol. 163, no. 11, 1 December 1999 (1999-12-01), pages 6275-6282

D4: JONES K R ET AL: "POLYCLONAL IN-VITRO PROLIFERATIVE RESPONSES FROM NONIMMUNE DONORS TO PLASMODIUM-FALCIPARUM MALARIA ANTIGENS REQUIRE UCHL1-POSITIVE MEMORY T CELLS" EUROPEAN JOURNAL OF IMMUNOLOGY, vol. 20, no. 2, 1990, pages 307-316

1. The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims is not new in the sense of Article 33(2) PCT.

D1 (p 37, last par. till p 42, par. 2 and examples 5 and 6) discloses a method for assessing immune response profiles of animal populations according to present claims 1-12.
Among the proteins tested, D1 (p 33, last par.) mentions proteases.
D1 (p 37, last par. till p 42, par. 2, examples 1-6, claims 1-10) additionally discloses a method for ranking the relative immunogenicity of a first protein and at least one additional protein according to present claims 14-25.
D1 (p 37, last par. till p 42, par. 2, examples 1-6, claims 11-23) furthermore discloses a method for ranking the relative immunogenicity of two proteins, wherein the second protein is a protein variant of the first protein, according to present claims 27-36.
D1 (p 16, last par.; p 18 par. 1; p 37, last par. till p 42, par. 2, example 7, claims 37-41) moreover discloses a method for determining the immune response of a test population against a test protein comprising determination of the level of exposure of said plurality of individuals to said test protein according to present claims 38-46.

D2 (p 281-288; fig. 2-5; table 1) discloses the I-MUNE® assay according to present claims 1-7, 14-19, 23-25, 27-33 and 35-36.
Among the proteins tested, D2 (p 281, co. 2, par. 3) mentions proteases according to present claims 11 and 12.
D2 (p 285, co. 2, par. 2 till p 286, co. 1, par.1) moreover discloses the application of structure index values for the creation of reduced immunogenicity proteins according to present claims 8-10, 20-22 and 34.
D2 (p 286, co. 2, par. 2) furthermore discloses, that a high structure value in the context of a low background rate suggests that humans are largely naive to the test proteins,

indicating, that the I-MUNE® assay is capable of determining the level of exposure of the tested individuals. D2 therefore also anticipates present claims 38-42.

2. The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 13, 26, 37 and 47 does not involve an inventive step in the sense of Article 33(3) PCT.

D1 (p 37, last par. till p 42, par. 2 and examples 5 and 6) is regarded as being the closest prior art to the subject-matter of claims 13, 26, 37 and 47 and discloses methods for assessing immune response profiles of animal populations by determining proliferation of $CD4^+$ T cells activated by antigen pulsed dendritic cells.

The subject-matter of claims 13, 26, 37 and 47 differs from this known method in that said method is validated by a different assay.

The problem to be solved by the present invention may therefore be regarded as providing a method according to D1, additionally validated by a lymphoproliferative assay on the basis of undifferentiated cell populations.

The solution, to use an assay based on peripheral blood mononuclear cells (PBMC) for validation of the I-MUNE® assay, directly derived from the individuals under study, cannot be considered as involving an inventive step (Article 33(3) PCT) for the following reasons.
Lymphoproliferative assays on the basis of PBMC in general are well known in the art. D3 (p 6276, co. 2, par. 4 and table II) discloses proliferation of PBMC in response to auto-antigens. PBMC proliferation could be detected not only in individuals showing symptoms of autoimmune disease (type I diabetes), but also in healthy controls. D3 therefor demonstrates that the assay is sensitive enough to detect not only proliferation of previously expanded T-cell sets (PBMC from autoimmune donors), but also from "naive donors".
Lymphoproliferative assays on the basis of PBMC have also been used for assessing proliferation of T-cells of donors to heterologous test proteins. D4 (p 308, co 2, par 2.4., table 1 and fig. 1) discloses proliferation of PBMC of nonimmune donors in response to whole protein malaria antigens.
The skilled person would therefore regard it as obvious to include such a PBMC based

lymphoproliferative assay for validation of a method according to D1.

Re Item VIII

Certain observations on the international application

1. **Claims 1, 14, 27 and 38** do not meet the requirements of Article 6 PCT in that the matter for which protection is sought is not clearly defined. The claims attempt to define the subject-matter in terms of the result to be achieved: "differentiating said dendritic cells to produce differentiated dendritic cells"
This merely amounts to a statement of the underlying problem, without providing the technical features necessary for achieving this result. The Applicant is therefore asked to replace said term with the method steps characterizing the differentiation process.

2. The following terms are vague and unclear and leave the reader in doubt as to the meaning of the technical features to which they refer, thereby rendering the definition of the subject-matter of said claim/s unclear, Article 6 PCT.

2.1. "structure values" in claims 4, 6, 7, 14, 17, 25, 27, 38 and 46: The Applicant is asked to amend said term by the definition given on p 27, last par.: "total variation distance to the uniform"

2.2. "peripheral blood mononuclear cell response assessment" in claims 13, 26, 37 and 47: The Applicant is asked to amend said term on basis of example 17, where such a peripheral blood mononuclear cell response assessment is described.

2.3. "pepset" in claims 14, 15, 27, 30, 38 and 39: The Applicant is asked to amend said term by the definition given on p 24, par. 5.